A STUDY OF NON-ALCOHOLIC FATTY LIVER DISEASE IN TYPE II DIABETES

Dissertation Submitted to

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GOVT. STANLEY MEDICAL COLLEGE & HOSPITAL
THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY
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MARCH 2009

CERTIFICATE

This is to certify that the dissertation titled "**A STUDY OF NON-ALCOHOLIC FATTY LIVER DISEASE IN TYPE II DIABETES MELLITUS**" is the bonafide original work of **DR. R.P. SENTHIL KUMAR** in partial fulfillment of the requirements for M.D. Branch – I (General Medicine) Examination of the Tamilnadu DR. M.G.R Medical University to be held in MARCH 2009. The Period of study was from MAY 2007 to AUGUST 2008.

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DECLARATION

I, **DR. R.P SENTHIL KUMAR,** solemnly declare that dissertation titled **"A STUDY OF NON-ALCOHOLIC FATTY LIVER DISEASE IN TYPE II DIABETES MELLITUS"** is a bonafide work done by me at Government Stanley Medical College and Hospital, Chennai during 2006-2007 under guidance and supervision of my Unit Chief **Prof.V.RUCKMANI.,M.D**., **Professor and Head of the Department of Medicine.**

This dissertation is submitted to Tamilnadu DR. M.G.R Medical University, towards partial fulfillment of requirement for the award of **M.D. Degree (Branch – I) in General Medicine.**

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Date : 25-12-2008

(DR.R.P.SENTHILKUMAR)

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INTRODUCTION

Non-alcoholic steato hepatitis (NASH) is a subset of non-alcoholic fatty liver disease (NAFLD). It is a disorder currently characterized by a constellation of histological abnormalities identified on liver biopsy that are similar to those seen in alcoholic liver disease but in patients who consume little or no alcohol. The prevalence of NASH is increasing in parallel with dramatic increases in obesity, sedentary life style and Type II Diabetes Mellitus.

Diabetes is a common metabolic disorder that affects a large number of people worldwide, the diabetic population is ever growing and it has now reached enormous proportions. Diabetes mellitus affects almost all systems in the body and it causes considerable morbidity and mortality. Diabetes mellitus, hyperlipidemia and obesity have been implicated as potential causes for the development of NAFLD and now newer risk factors have been proposed.

A plethora of case series of NAFLD have been reported over the past few years but whether this indicates a true increase in prevalence or simply an increased awareness of this disorder is unclear.

Stanley Medical College is located in Chennai and caters to the medical needs of a large diabetic population. Many cases of Diabetes mellitus also have chronic liver disease; some of them do not have a history of significant alcohol consumption, so we thought that these cases might represent a sample of what is called cryptogenic cirrhosis.

A significant proportion of patients previously thought to have cryptogenic cirrhosis share many of the clinical and demographic features of nonalcoholic fatty liver disease, suggesting that the etiology of their cirrhosis may be unrecognized NAFLD.

So we conducted this study to evaluate the prevalence and general characteristics of Non alcoholic Fatty Liver Disease in type II diabetics with a motive to provide some information that might be useful for future reference and to evaluate the impact of this disease on persons belonging to this geographical region.

AIM OF THIS STUDY

1. To find out the Prevalence and General characteristics of Non Alcoholic Fatty Liver Disease in persons with Type 2 Diabetes Mellitus attending outpatient clinic in Stanley Medical College.
2. To assess the different clinical presentations of Non Alcoholic Fatty Liver Disease in Type 2 Diabetes Mellitus patients.
3. To assess the relationship between Body Mass Index and Non Alcoholic Liver Disease in Type 2 Diabetes Mellitus.
4. To correlate the results of Liver Function Tests with Ultrasonographic evidence of fatty liver in Type 2 Diabetes Mellitus.
5. To correlate the results of fasting Lipid Profile with Ultrasonographic evidence of fatty liver in Type 2 Diabetes Mellitus.

REVIEW OF LITERATURE

The liver plays a pivotal role in the metabolism of carbohydrates and lipids; affection of liver is common in diabetes. Sometimes liver disease may give rise to abnormalities in glucose homeostasis, and finally certain diseases of liver might be present coincidentally with diabetes.[1]

LIVER DISEASE IN DIABETES MELLITUS

1. Liver disease occurring as a consequence of diabetes mellitus
    - Glycogen deposition
    - Steatosis and nonalcoholic steatohepatitis(NASH)
    - Fibrosis and cirrhosis
    - Biliary disease, cholelithiasis, cholecystitis
    - Complications of therapy of diabetes (cholestatic and necroinflammatory)
2. Abnormalities of glucose homeostasis occurring as a complication of liver disease can be present in
    - Hepatitis
    - Cirrhosis
    - Hepatocellular carcinoma
    - Fulminant hepatic failure

3. Liver disease occurring coincidentally with diabetes mellitus and abnormalities of glucose homeostasis.
    - Hemochromatosis
    - Glycogen storage diseases
    - Autoimmune biliary disease

Hepatic fat accumulation is a well recognized complication of diabetes with a reported frequency of 40 – 70 %. Unfortunately, associated obesity is frequently occurring confounding variable. Type I diabetes is not associated with fat accumulation if glycemia is well controlled, but type 2 diabetes may have a 70 % correlation regardless of blood glucose control.[1]

HISTORICAL ASPECTS

Jurgen Ludwig, a Mayo Clinic pathologist, popularized the term "Non alcoholic steatohepatitis" in a paper published in 1980.[2]

In 1952 Zelman described liver biopsy findings in 19 obese men that included steatosis and varying degrees of inflammation and fibrosis.[3]

These early studies set the stage for the Ludwig series that convinced many in the field that NASH was a potentially serious disorder that was truly unrelated to alcohol consumption and could no longer be ignored.

NOMENCLATURE

Nonalcoholic fatty liver disease refers to a broad spectrum of liver disease ranging from steatosis (bland fatty infiltration of hepatocytes) to nonalcoholic steatohepatitis (steatosis plus inflammation, necrosis or fibrosis) to cirrhosis and, in some patients, to end-stage liver disease and hepatocellular carcinoma. These facts have been in documented in studies done by Lee R .G et al (1989)[4] and Powell E .E et al [5](1990).

EPIDEMIOLOGY

RISK FACTORS FOR NAFLD

The **most common underlying risk factor** for the development of NASH is the presence of **insulin resistance**. NASH is found to occur equally in both genders. Hyperlipidemia, typically hypertriglyceridemia is associated with NAFLD.[6-12]

CONDITIONS ASSOCIATED WITH NAFLD [6-12]

INSULIN RESISTANCE

- Obesity
- Sedentary lifestyle
- Type 2 diabetes
- Hypertriglyceridemia
- Hypertension

DRUGS

- Tamoxifen
- Corticosteroids
- Amiodarone
- Estrogens
- Calcium-channel blockers

TOXINS

- Extensive exposure to volatile hydrocarbons

DIETARY ABNORMALITIES

- Carbohydrate excess (e.g., dietary, total parenteral nutritional)
- Protein deficiency
- Rapid weight loss
- Vitamin B12 deficiency

ALTERED SMALL ANATOMY

- Obesity surgery with blind loop of small bowel
- Small bowel diverticula
- Short gut

METABOLIC DISEASES (RESULTING IN NASH-LIKE HISTOLOGY)

- Hyperbetalipoproteinemis

- Abetalipoproteinemia
- Wilson's disease
- Lipodystrophies
- Andersen's disease
- Weber-Christian syndrome
- Mauriac syndrome

INFECTIONS

- Chronic hepatitis C (usually genotype 3)
- AIDS
- Bacillus cereus infection

PREVALENCE OF NAFLD IN ADULTS

The best estimates based on currently available data indicate that about 20 % of adults in the USA have Non-Alcoholic Fatty Liver Disease(NAFLD) and about 2-3 % of all adults have Non-Alcoholic Steato-Hepatitis(NASH)**.**[13, 14]

NAFLD(including NASH) : 20 %

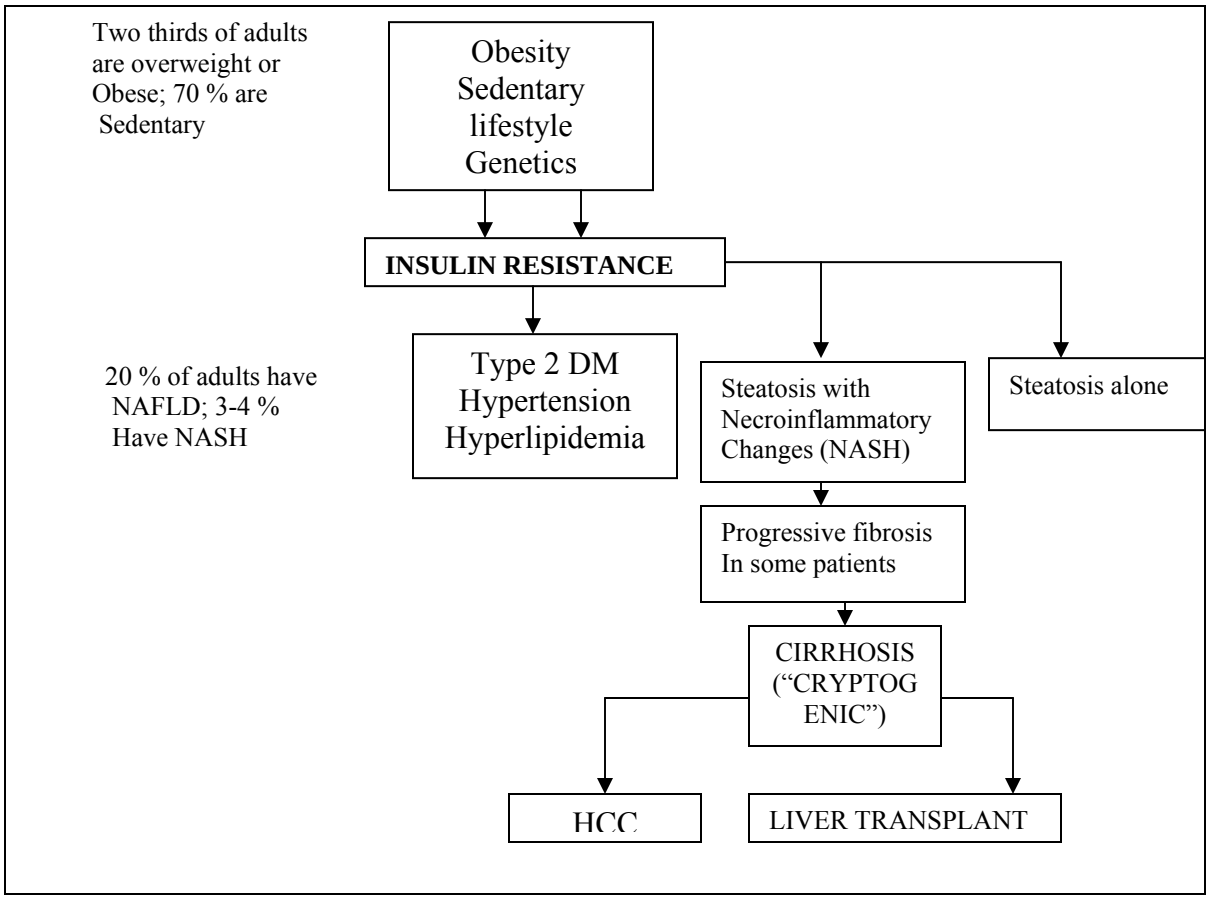
NASH : 3-4 %

NASH with fibrosis : 1 %

PREVALENCE OF NAFLD IN CHILDREN

The full spectrum of NAFLD is found in children.[15] One study in Japan of 810 children aged 4-12 years old demonstrated the presence of sonographically detectable NAFLD in 2-6 % and its presence correlated with obesity.[16]

PROGRESSION OF NAFLD



CLINICAL MANIFESTATIONS

CLINICAL FEATURES

NAFLD is usually asymptomatic[14], although fatigue and discomfort in the right upper quadrant of the abdomen may be reported.[17] The majority (56%-79%) of patients are overweight (body mass index [BMI] >25 kg/m^2), and one-third have metabolic syndrome.[18,19,20] Lean patients (BMI 25 kg/m^2) usually have at least one metabolic risk factor. Hepatomegaly may be present, although signs of chronic liver disease are uncommon.[17, 21]

Hepatomegaly is the only physical finding in most patients. Acanthosis nigricans may be found in children with nonalcoholic fatty liver disease.[18, 22] Findings of chronic liver disease and diminished number of platelets suggest that advanced disease with cirrhosis is present. A high proportion of patients with cryptogenic cirrhosis share many of the clinical and demographic features of patients with nonalcoholic fatty liver disease,[23] suggesting that their cryptogenic cirrhosis is unrecognized nonalcoholic fatty liver disease.

Common symptoms and signs of 400 subjects with NAFLD (Data from the NAFLD clinic at Virginia Commonwealth University, previously unpublished data).[24]

Symptoms and signs	NAFLD (N=75) %	NASH (N=325) %
Asymptomatic	60	55
Fatigue	30	45
Pruritis	2	4
Rt. upper quadrant discomfort	30	32
Edema	4	5
hepatomegaly	22	28
Stigmata of chronic liver disease	8	10
Diabetes	45	50
Hypertension	60	65
Obesity	65	60

LABORATORY ABNORMALITIES

Mildly to moderately elevated serum levels of aspartate aminotransferase, alanine aminotransferase, or both are the most common and often the only laboratory abnormality found in patients with nonalcoholic fatty liver disease. The ratio of aspartate aminotransferase to alanine aminotransferase is usually less than 1, but this ratio increases as

fibrosis advances, leading to a loss of its diagnostic accuracy in patients with cirrhotic nonalcoholic fatty liver disease.[25] Serum alkaline phosphatase, gamma glutamyltransferase, or both are above the normal range in many patients, although their degree of elevation is less than seen in alcoholic hepatitis. An ALT or AST value >300 IU/L should raise the suspicion of alternate pathology.[26] The degree of abnormality is usually moderate and does not exceed 2-3 times the upper limit of normal values. Unfortunately, none of these tests are sensitive or specific enough to establish a diagnosis of NAFLD with great accuracy.

Other abnormalities including hypoalbuminemia, a prolonged prolonged prothrombin time, and hyperbilirubinemias, may be found in patients with cirrhotic stage nonalcoholic fatty liver disease.

Ferritin levels are increased in 20%-50% of patients, and elevated transferring saturation (>55%) is present in 5-10%.[27]

Autoantibodies are identified in 23%-36% of NAFLD patients and are associated with more advanced fibrosis.[28, 29]

However, in studies of subjects with persistently elevated ALT values without an obvious explanation, NAFLD was found in only 70-80% of cases and 20-30% of subjects were found to have an alternate cause for their

elevated liver enzymes.[30] Of note, 5-9% of subjects had a normal liver despite a complete evaluation.

IMAGING STUDIES

On **Ultrasonography**, fatty infiltration of the liver produces a diffuse increase in echogenicity as compared with that of the kidneys. Regardless of the cause, cirrhosis has a similar appearance on ultrasonography. **Ultrasonography has a sensitivity of 89 percent and a specificity of 93 percent in detecting steatosis and a sensitivity and specificity of 77 percent and 89 percent, respectively, in detecting increased fibrosis**.[31]

Fatty infiltration of the liver produces a low-density hepatic parenchyma on Computed Tomographic (CT) scanning. Steatosis is diffuse in most patients with nonalcoholic fatty liver disease, but occasionally, it is focal. Sonography of fatty liver may be varied depending on the amount of fat and whether deposits are diffuse or focal.[32]

Diffuse steatosis may be: [33]

Mild: minimal diffuse increase in hepatic echogenicity; normal visualization of diaphragm and intrahepatic vessel borders.

Moderate: moderate increase in hepatic echogenicity; slightly impaired visualization of intrahepatic vessels and diaphragm.

Severe: marked increase in echogenicity; poor penetration of the posterior segment of right lobe of liver and poor or non-visualization of the hepatic vessels and diaphragm.

Sonographic features of focal fatty changes are:

Focal fat may show rapid change with time both in appearance and resolution, it does not alter the course or caliber of regional blood vessels and does not produce contour abnormalities, and the preferred site for both focal fat deposition and focal sparing is the area anterior to the portal vein at the porta hepatis. Sometimes focal fat may produce geographic map-like boundaries.

CT imaging of the liver produces a more sensitive method for the non-invasive diagnosis of NAFLD. Hepatic steatosis decreases the CT attenuation of the liver. When the hepatic parenchymal attenuation is 10 or more Houndsfield units lower than the spleen on a non-contrast-enhanced scan, a diagnosis of hepatic steatosis can be made. When intravenous contrast is administered, the hepatic enhancement lags behind the spleen and the liver-to-spleen attenuation differential exceeds 20 Houndsfield units. These features allow hepatic steatosis to be defined with a 76% positive predictive value.[34]

Magnetic resonance spectroscopy allows a quantitative assessment of fatty infiltration of the liver, [35] and a minimum of 5%-10% steatosis by weight is considered a requirement for the diagnosis of NAFLD.

The sensitivity of each imaging method increases with the degree of fatty infiltration, with at least 33% steatosis being optimal for detection.[34]

The combination of steatosis, infiltration by mononuclear cells or polymorphonuclear cells (or both), and hepatocyte ballooning and spotty necrosis is known as nonalcoholic steatohepatitis. Most patients with this type of non alcoholic fatty liver disease have some degree of fibrosis, whereas Mallory's hyaline may or may not be present. The severity of steatosis can be graded on the basis of the extent of involved parenchyma.[36] A system that unifies the lesions of steatosis and necroinflammation into a "grade" and those of the types of fibrosis into a "stage" has been recently proposed.[36]

PATHOGENESIS[37]

The pathogenesis of nonalcoholic fatty liver diseases has remained poorly understood since the earliest description of the disease. Much current thinking remains hypothetical, since the mechanism or mechanisms are still being worked out. It is not yet understood why simple steatosis develops in some patients, whereas steatohepatitis and progressive disease develop in

others; differences in body-fat distribution or antioxidant systems, possibly in the context of a genetic predisposition, may be among the explanations.

A net retention of lipids within hepatocytes, mostly in the form of triglycerides, is a prerequisite for the development of nonalcoholic fatty liver disease.

Insulin resistance (owing to inhibition of tumor necrosis factor α [TNF- α], Rad, PC-1, eptin, and fatty acids) **leads to the accumulation of fat in hepatocytes by two main mechanisms: lipolysis,** which increases circulating fatty acids, and **hyperinsulinemia.** Increased uptake of fatty acids by hepatocytes leads to mitochondrial β-oxidation overload, with the consequent accumulation of fatty acids within hepatocytes. Fatty acids are substrates and inducers of the microsomal lipoxygenases cytochrome P-450 2E1 and 4A.[38,39] The level of cytochrome P-450 2E1 is invariably increased in the liver of patients with steatohepatitis and may result in the production of free oxygen radicals capable of inducing lipid peroxidation of hepatocyte membranes.[38] Hyperinsulinemia resulting from insulin resistance increases the synthesis of fatty acids in hepatocytes by increasing glycolysis and favors the accumulation of triglycerides within hepatocytes by decreasing hepatic production of apolipoprotein B-100.

Micrososmal ω-oxidation of fatty acids generates dicarboxylic fatty acids, which are further degraded by peroxisomal β-oxidation. Peroxisomal β-oxidation generates chain-shortened acyl-coenzyme A. Very-long-chain fatty acids are converted to acyl-coenzyme A by the action of acyl-coenzyme A synthetase. Acyl-coenzyme A serves as a substrate for peroxisomal oxidation, but if left unmetabolized, it functions as a PPAR- α ligand. PPAR- α controls the induction of genes involved in microsomal, peroxisomal, and mitochondrial fatty-acid systems in liver, and it may also promote hepatic synthesis of uncoupling protein-2. The role of this protein in the pathogenesis of nonalcoholic fatty liver disease remains uncertain. It may help inhibit hepatocyte apoptosis, but it may also increase the vulnerability of fatty hepatocytes to subsequent injury when exposed to secondary insults such as endotoxin or TNF- α.[40, 41]

Mitochondrial reactive oxygen species promote progression from steatosis to steatohepatitis[42, 43] and fibrosis by three main mechanisms: lipid peroxidation, cytokine induction, and Fas ligand induction. Reactive oxygen species trigger lipid peroxidation, which causes cell death and releases malondialdehyde (MDA) and 4-hydrxynonenal (HNE). MDA and HNE cause cell death; cross-link proteins, leading to the formation of

Mallory's hyaline; and activate stellate cells, promoting collagen synthesis. HNE has chemotactic activity for neutrophils, promoting tissue inflammation. Reactive oxygen species also induce the formation of the cytokines TNF- α, transforming growth factor β (TGF- β), and interleukin-8. TNF- α and TGF- β cause caspase activation and hepatocyte death. TGF- β activates collagen synthesis by stellate cells and activates tissue transglutaminase, which cross-links cytoskeletal proteins, promoting the formation of Mallory's hyaline. Interleukin-8 is a potent chemoattractant for human neutrophils. The TNF- α induced by reactive oxygen species further impairs the flow of electrons along the respiratory chain in mitochondria. Mitochondrial reactive oxygen species can deplete hepatic antioxidants, allowing accumulation of more reactive oxygen species.[42, 43] Mitochondrial reactive oxygen species cause expression of the Fas ligand in hepatocytes, which normally express the membrane receptor Fas. The Fas ligand on one hepatocyte can then interact with Fas on another hepatocyte, causing fractional killing.

Insulin resistance is the most reproducible factor in the development of nonalcoholic fatty liver disease.[44, 45] The molecular pathogenesis of insulin resistance seems to be multifactorial, and several molecular targets involved in the inhibition of insulin action have been identified. **Insulin resistance**

leads to fat accumulation in hepatocytes by two main mechanisms; lipolysis and hyperinsulinemia.

Clinically significant amounts of dicarboxylic acids, which are potentially cytotoxic, can be formed by microsomal ω-oxidation. This pathway of fatty acid metabolism is closely related to mitochondrial β-oxidation and peroxisomal β-oxidation. Deficiency of the enzymes of peroxisomal β-oxidation has been recognized as an important cause of microvesicular steatosis and steatohepatitis.[40] Deficiency of acyl-coenzyme A oxidase disrupts the oxidation of very-long-chain fatty acids and dicarboxylic acids, leading to extensive microvesicular steatosis and steatohepatitis. Loss of this enzyme also causes sustained hyperactivation of peroxisome-proliferator-activator receptor α (PPAR- α), leading to transcriptional up-regulation of PPAR- α-regulated genes. PPAR- α has been implicated in promoting hepatic synthesis of uncoupling protein-2, which is expressed in the liver of patients with nonalcoholic fatty liver disease.

Increased intrahepatic levels of fatty acids provide a source of oxidative stress, which may in large part, be responsible for the progression from steatosis to steatohepatitis to cirrhosis. Mitochondria are the main cellular source of reactive oxygen species, which may trigger steatohepatitis and fibrosis by three main mechanisms; lipid peroxidation, cytokine

induction, and induction of Fas ligand. Patients with steatohepatitis have ultrastructutal mitochondrial lesions; including linear crystalline inclusions in megamitochondria.[46] This mitochondrial injury is absent in most patients with simple steatosis and in healthy subjects. Patients with steatohepatitis slowly resynthesize ATP in vivo after a fructose challenge, which causes acute hepatic ATP depletion.[47] This impaired ATP recovery may reflect the mitochondrial injury found in patients with steatohepatitis.[46]

Thus, although symptoms of liver disease rarely develops in patients with fatty liver who are obese, have diabetes, or have hyperlipidemia, the steatotic liver may be vulnerable to further injury when challenged by additional insults. This has led to the presumption that **progression from simple steatosis to steatohepatitis and to advanced fibrosis results from two distinct events.**[48] First, **insulin resistance** leads to the accumulation of fat within hepatocytes, and second, **mitochondrial reactive oxygen species** cause lipid peroxidation, cytokine induction, and the induction of Fas ligand.

DIAGNOSIS

The diagnosis of nonalcoholic fatty liver disease is usually suspected in persons with asymptomatic elevation of aminotransferase levels, radiologic finds of fatty liver, or unexplained persistent hepatomegaly. The clinical diagnosis and liver tests have a poor predictive value with respect to histologic involvement.[49] Imaging studies, although of help in determining the presence and amount of fatty infiltration of the liver, cannot be used to accurately determine the severity of liver damage.

Liver biopsy is considered as best method for the detection of hepatic steatosis and it can also detect steatohepatitis.

The diagnosis of nonalcoholic fatty liver disease requires the exclusion of alcohol abuse as the cause of liver disease; a daily intake as low as 20 gm in females and 30 gm in males may be sufficient to cause alcohol-induced liver disease in some patients (350 ml [12 oz] of beer, 120 ml [4 oz] of wine, and 45 ml [1.5 oz] of hard liquor each contain 10 gm of alcohol).[50] Other causes, such as viruses, autoimmune responses, metabolic or hereditary factors, and drugs or toxins, should be ruled out. The decision on how extensive the serologic workup should be individualized.

Even though liver biopsy is considered to be the best, some advocate that there are several drawbacks in using liver biopsy for this purpose. This

procedure is invasive, costly, and prone to complications, some minor, such as pain, others more severe with a recorded risk of death of 0.01%. Notably, just as is the case in other chronic liver disease, there is considerable sampling variability (40% for fibrosis staging), and a high intra and inter-pathologist variability.[51] Most importantly, the number of patients at risk for NAFLD is high enough that liver biopsy is not a practical and efficient tool for identifying those at risk of advanced fibrosis. Indeed an estimated 15-20% of the Western European population has steatosis while more than half of Americans are overweight of obese.

So the diagnostic workup needs to be individualized and decisions taken accordingly.

Some newer methods are now emerging for the diagnosis of hepatic steatosis and steatohepatitis such as the H magnetic resonance spectroscopy and the fibrosure test for the detection of fibrosis. These tests may in course of time serve as a better noninvasive method for the detection of NAFLD

NATURAL HISTORY

The natural history of nonalcoholic fatty liver disease is not well defined, but it seems to be determined by the severity of histological damage. In five series, 54 of 257 patients with nonalcoholic fatty liver

disease underwent liver biopsy during an average follow-up of 3.5 to 11 years.[4, 5] Of these patients, 28 percent had progression of liver damage, 59 percent had essentially no change, and 13 percent had improvement or resolution of liver injury. Progression from steatosis to steatohepatitis and to more advanced fibrosis[4, 5] or cirrhosis[4, 5] has been recognized in several cases. Some of the few deaths occurred among the 257 patients were liver-related, including one from hepatocellular cancer. Thus, many patients with nonalcoholic fatty liver disease have a relatively benign course, whereas in some others, the disease progresses to cirrhosis and its complications.

Patients found to have pure steatosis on liver biopsy seem to have the best prognosis within the spectrum of nonalcoholic fatty liver disease, whereas features of steatohepatitis of more advanced fibrosis are associated with a worse prognosis. In one study,[52] progression of liver fibrosis occurred only in patients with necrosis and inflammatory infiltration on liver biopsy. In another study, 36 percent of patients with nonalcoholic fatty liver disease died after a mean follow-up of 8.3 years, liver-related diseases were the second most common cause of death, exceeded only by cancer. Some data suggest that the coexistence of steatosis with other liver diseases, such as hepatitis C virus infection, could increase the risk of progression of the liver disease.[53] The natural history of cirrhosis resulting from nonalcoholic fatty

liver disease has not been completely defined. In a recent study, [54] only 2.9 percent of 545 liver-transplantation procedures performed in a single center were for end-stage steatohepatitis.

This suggests that although nonalcoholic fatty liver disease is common, only a minority of patients will require liver transplantation.

TREATMENT

Many clinical trials on to find out an effective method of treatment of Nonalcoholic Fatty Liver Disease, many treatment options have been suggested and they are:

1. **Treatment of associated disorders**

   Gradual weight loss, Control of diabetes, Control of dyslipidemia

2. **Potential pharmacological approaches**

   Improved insulin resistance

   Metformin, Thiazolinediones: Pioglitazone

   Improved dyslipidemia

   Clofibrate, Gemfibrozil, Atorvastatin, Probucol

   Antioxidants

   Tocopherol, Tocopherol/ Ascorbic acid, Betaine, Ursodeoxycholic acid, S-adnosyl methionine.

3. **Liver transplantation**

MATERIALS AND METHODS

MATERIALS AND METHODS

INCLUSION CRITERIA

Patients who were diagnosed to have Type 2 Diabetes Mellitus, for more than 3 years duration, belonging to both sexes and with age of more than 40 years attending Diabetology Out-patient Department of Stanley Medical College were included in the study.

EXCLUSION CRITERIA

Patients with history of alcohol consumption for any duration of time were excluded.

Persons with previous history of jaundice, ascites and signs of liver cell failure were excluded.

Persons who tested positive for Hepatitis B serology by Elisa or by card test were excluded.

Patients with history of intake of Methotrexate, Amiodarone, Glucocorticoids, Synthetic Estrogens, Nucleoside Analogues (ddI, AZI) were excluded.

Persons with history of major abdominal surgeries were excluded.

Persons with history of Chronic Renal Failure and severe Ischemic Heart Disease were excluded from the study.

Patients with history of Ketoacidosis or with a history of prolonged treatment with insulin were excluded.

The Study Population was derived from the patients attending the Diabetology Outpatient Department of Stanley Medical College from January 2007 to August 2008.

A detailed history was taken regarding the Duration of Diabetes, Symptoms pertaining to the Hepatobiliary System.

History of medications was obtained in detail.

History of alcohol consumption was recorded and any person with history of alcohol use was excluded from the study population. Any history of previous abdominal surgeries such as Jejuno- Ileal Bypass, Gastrectomy was recorded.

Women were enquired about oral contraceptive or hormonal use.

A detailed Clinical Examination of all systems was made and signs of Liver Cell Failure, Organomegaly, Ascites were looked for.

The patient's Height & Weight were recorded & Body Mass Index was calculated.

BMI is defined as weight in kilograms divided by height in meter's squared.

Patients were classified according to BMI as follows:

Underweight: BMI <18.5 kg/m^2

Normal weight: BMI 18.5 to 24.9 kg/m^2

Overweight: BMI 25 to 29.9 kg/m^2

Obese: BMI >30 kg/m^2

Blood pressure measurements were taken.

THE LABORATORY INVESTIGATIONS DONE INCLUDED.

A Complete Blood Count.

Urine for Albumin, Sugar and Deposits.

Blood sugar: Random, Fasting & Post-Prandial.

Blood Urea and Serum Creatinine.

Serum Electrolytes – Sodium & Potassium.

LIVER FUNCTION TESTS

SGOT (Normal value 5 to 35 IU/L)

SGPT (Normal value 5 to 35 IU/L)

Serum Alkaline Phosphatase (Normal value 60 to 170 IU/L)

Serum Total Bilirubin (Normal value < 1 mg/dl)

Serum Total Proteins.

FASTING LIPID PROFILE

The fasting lipid profile was done after a minimum of 12 hours of overnight fasting and the following tests were done.

Serum Total Cholesterol.

Serum Triglycerides (TGL).

Serum High Density Lipoprotein (HDL)

Serum Low Density Lipoprotein (LDL) was calculated using the Friedwald formula:

LDL-C = Total Cholesterol – HDL C – (Triglyceride/5).

Ultrasonogram of Abdomen was done with particular focus on the liver.

The presence of diabetes was defined according to the WHO CRITERIA AS:

Symptoms of diabetes, plus Random Blood concentration more than 200 mg/dl.

Fasting plasma glucose more than 126 mg/dl. (Fasting is defined as no caloric intake for at least 8 hours)

Two-hour plasma glucose more than 200 mg/dl during an oral Glucose Tolerance Test. (This test should be done using a glucose load containing the equivalent of 75 gm of anhydrous glucose dissolved in water).

Type 2 DM subjects were defined as those with previous physician-diagnosed diabetes in whom hyperglycemia had been controlled for one year or more with oral hypoglycemic agents and diet, with absence of history of ketoacidosis initially, or during the course of the disease.[55]

IMAGING STUDY

Steatosis was defined as the presence of an Ultrasonographic pattern consistent with **"BRIGHT LIVER,"** with evident Ultrasonographic contrast between hepatic and renal parenchyma, vessel blurring, focal sparing, and narrowing of the lumen of the hepatic veins, according to international guidelines.[31] The upper limit of normal liver size was 15 cm in the longitudinal plane, any measurement above this was considered hepatomegaly. Mild hepatomegaly was defined as liver size > 15 – 18 cm in the longitudinal plane.

The presence of steatosis was graded from mild to severe and for calculation purposes all grades were taken as positive fatty liver.

All the images were reviewed by another radiologist to minimize observer errors.

The L&T Ultrasound machine used had a 3.5 MHz probe.

STATISTICAL ANALYSIS

Statistical analysis of the data obtained from the study was done using the **'z' test or 'normal' test** to compare the mean values of two groups of participants. The **chi-square test** was used to compare the prevalence between the two groups. The calculations were done for 5% level of significance. ($P = 0.05$).

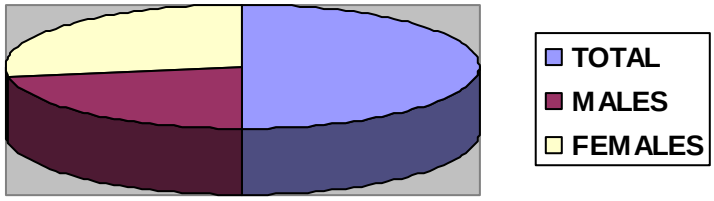
RESULTS AND OBSERVATIONS

RESULTS AND OBSERVATIONS

A total of 109 patients diagnosed with type 2 Diabetes Mellitus for 3 years and more were included in this study after applying the selection criteria. Most of them belonged to the low and middle socio-economic groups.

Out of the 109 participants 60 were females and 49 were males.

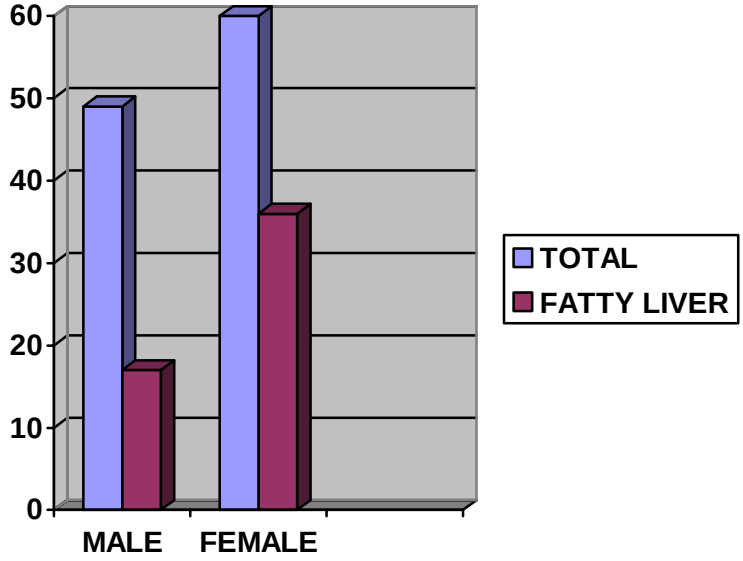
SEX DISTRIBUTION



The age of the participants varied from 40 to 75 years and the mean age was 52.45 ± 7.15 years.

Out of the total 109 participants 53 persons (48.62 %) had ultrasonographically detected fatty liver. Most of them had moderate or severe steatosis ultrasonogram wise. They were called as the NAFLD (Non Alcoholic Fatty Liver Disease) group. Of these 53 persons 36 were females and 17 were males.

SEX-WISE PREVALENCE



	Female(total 60)	Male (total 59)	P value
NAFLD in USG(53)	36 (60 %)	17 (34.69 %)	< 0.05
Normal USG(56)	24 (40 %)	32 (65.31 %)	

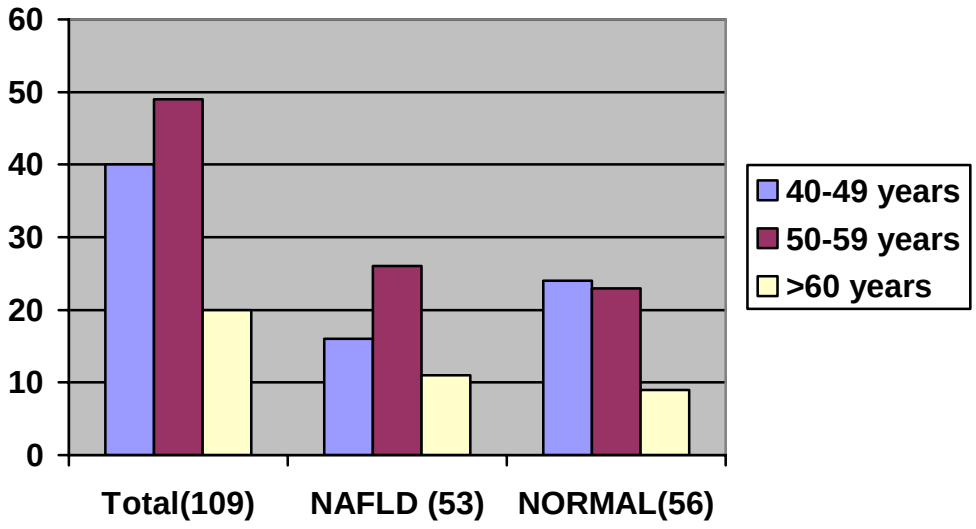
The duration of Diabetes varies from 3 to 20 years in the study group with a mean value of 5.48 ± 3.57 years.

The mean duration of Diabetes in the fatty liver group was 5.47 ± 3.19 years as compared to 5.48 ± 3.94 years in the normal liver group. There was no significant difference between the NAFLD group and the normal group duration wise (P value >0.05).

The age wise distribution of patients with and without fatty liver in Ultrasonogram is as follows.

Age group	Total(109)	NAFLD(53)	Normal(56)
40-49 years	40	16 (30.1 %)	24 (42.8 %)
50-59 years	49	26 (49.05 %)	23 (41.07 %)
>60 years	20	11 (20.75 %)	9 (16.98 %)

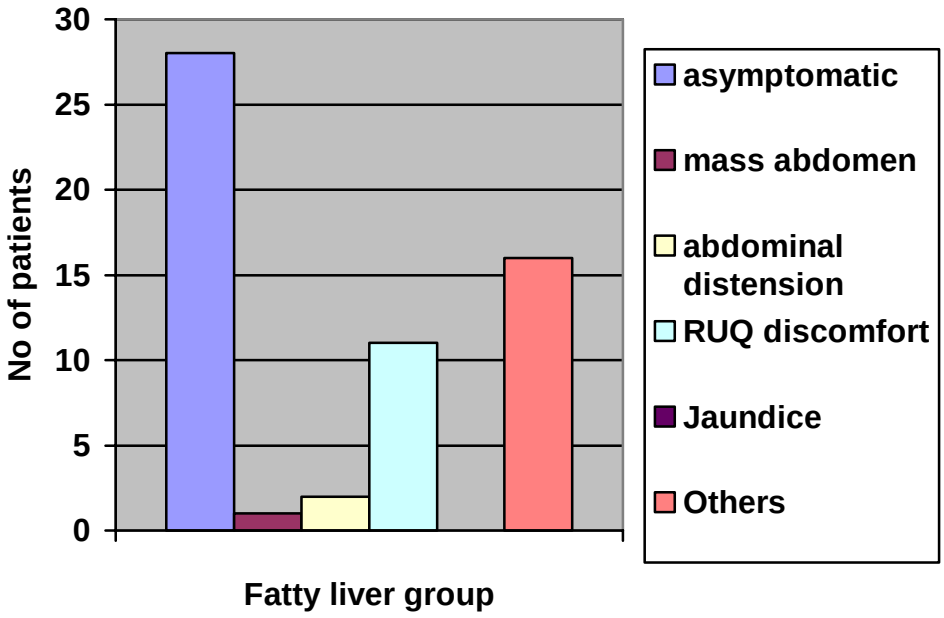
AGE WISE PREVALENCE



CLINICAL FEATURES

Most of the persons with fatty liver were asymptomatic, i.e. 28 out of total 53. The next common symptom was right upper quadrant discomfort, which was present in 11 out of 53 patients, 2 persons in the fatty liver group had complaint of abdominal distension, and no patient had the complaint of jaundice, 16 persons had a feeling of generalized weakness and malaise.

SYMPTOMS



Clinical examination of abdomen revealed hepatomegaly in 6 patients with fatty liver and 1 patient in the normal group. No patient in both groups had splenomegaly or ascites. Ultrasonography showed hepatomegaly in 9

out of 53 persons with fatty liver compared to 1 out of 56 persons in the normal liver group.

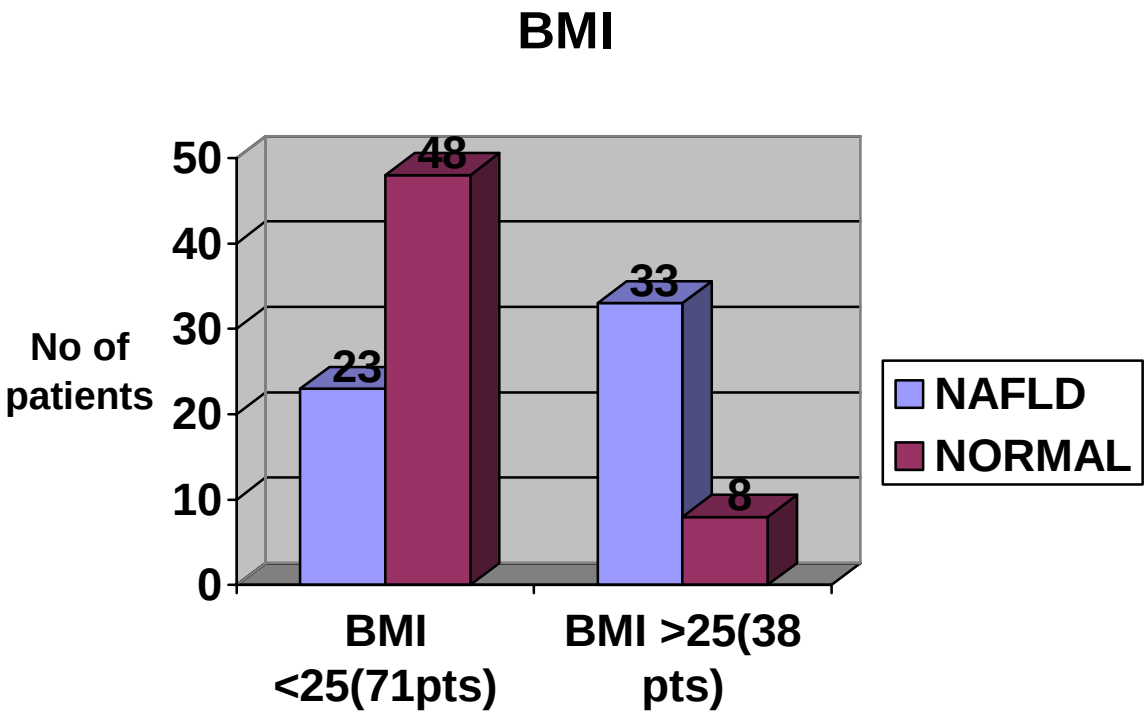
BODY MASS INDEX

The Body Mass Index varied (BMI) from 17 to 37 kg/m^2 with a mean Body Mass Index of 23.60 ± 3.17 kg/m^2. A BMI of 25 kg/m^2 was taken as a cut-off between overweight and obese, 71 persons had a BMI below 25 kg/m^2 and 38 persons had a BMI of above 25 kg/m^2. Only 4 persons had a BMI of more than 30 kg/m^2 and all of them had fatty liver. Out of the patients with a BMI of more than 25 kg/m^2 (total 38) 30 persons had fatty liver detected in ultrasonogram. In the low BMI group (total 71) 27 persons had Ultrasonographically detected fatty liver.

Mean BMI values:

NAFLD group	Normal group	P value
24.97 ± 3.54 kg/m^2	22.29 ± 2.05 kg/m^2	< 0.05

BMI (kg/m^2)	NAFLD GROUP (53)	NORMAL USG (56)
<25 (71)	23 (32.39 %))	48 (67.61 %)
>25 (38)	30 (78.94 %)	8 (21.06 %)



LABORATORY INVESTIGATIONS

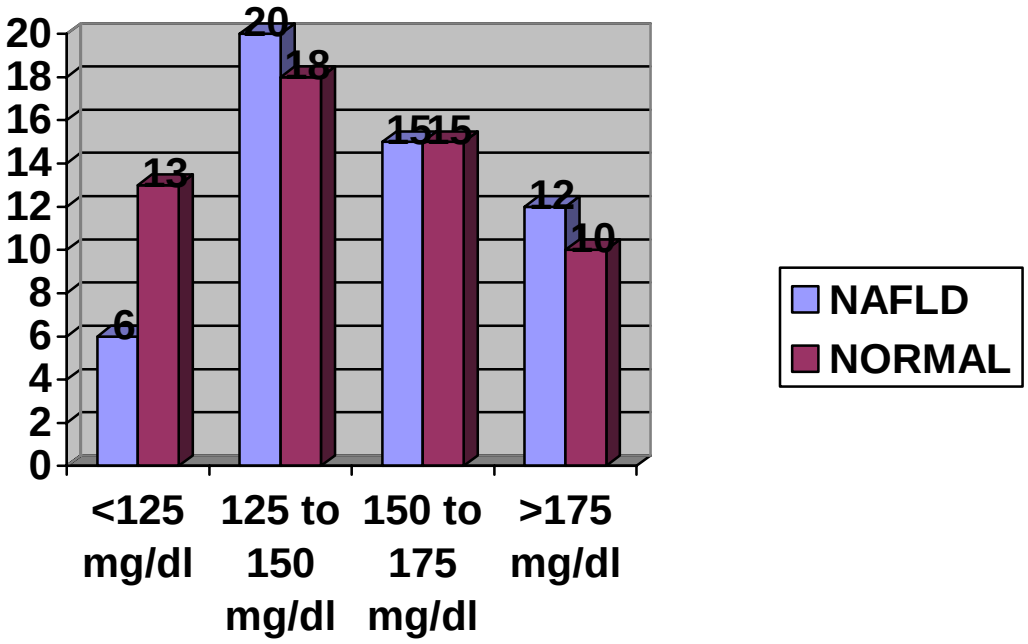
BLOOD SUGAR

All the patients had a random, fasting and postprandial blood sugar estimation done.

The number of patients with and without fatty liver in the different fasting blood sugar categories was as follows.

FBS (mg/dl)	TOTAL	FATTY LIVER	NORMAL USG
< 125	19	6	13
125 TO 150	38	20	18
150 TO 175	30	15	15
> 175	22	12	10

FASTING BLOOD SUGAR WISE DISTRIBUTION



The mean fasting blood sugar in the above two categories are:

NAFLD group	Normal group	P value
156.19 ± 36.53 mg/dl	146.67 ± 32.38 mg/dl	> 0.05

LIVER FUNCTION TESTS

The liver function tests done included the Serum Trasnsaminases, Serum Alkaline Phosphatase, Serum Total Bilirubin and Total Proteins.

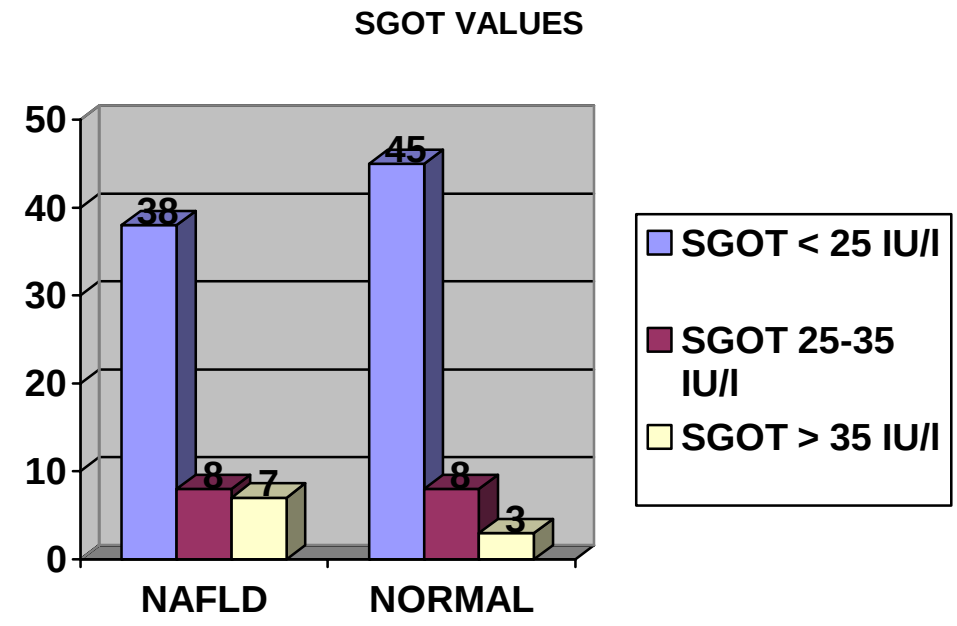
The normal value of serum transaminases is 5 to 35 IU/l. The normal value of Serum alkaline Phosphatase is up to 150 IU/l. The participants were categorized into a low Transaminase level group of 25 IU/l or below and a high normal and increased Transaminase level group with a value of more than 25 IU/l.

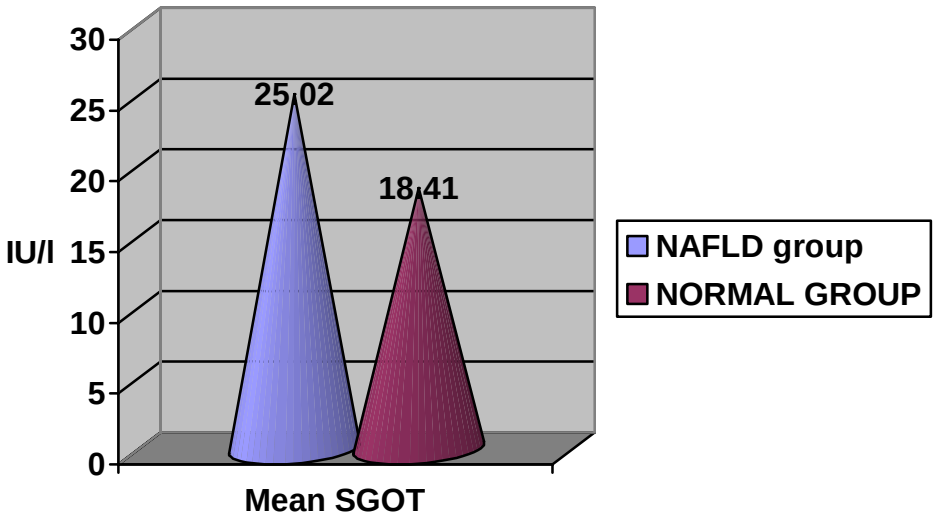
SGOT LEVELS:

SGOT levels	Total (109)	NAFLD group (53)	Normal group (56)
< 25 IU/l	83	38 (71.69 %)	45 (80.35 %)
25-35 IU/l	16	8 (15.09 %)	8 (14.28 %)
> 35 IU/l	10	7 (13.20 %)	3 (7.14 %)

Mean SGOT values:

NAFLD group	Normal group	P value
25.02 ± 20.64 IU/l	18.41 ± 11.97 IU/l	< 0.05


SGOT VALUES
50
40
30
20
10
0
38
8
7
45
8
3
NAFLD
NORMAL
SGOT < 25 IU/l
SGOT 25-35 IU/l
SGOT > 35 IU/l


30
25
20
IU/l 15
10
5
0
25.02
18.41
Mean SGOT
NAFLD group
NORMAL GROUP

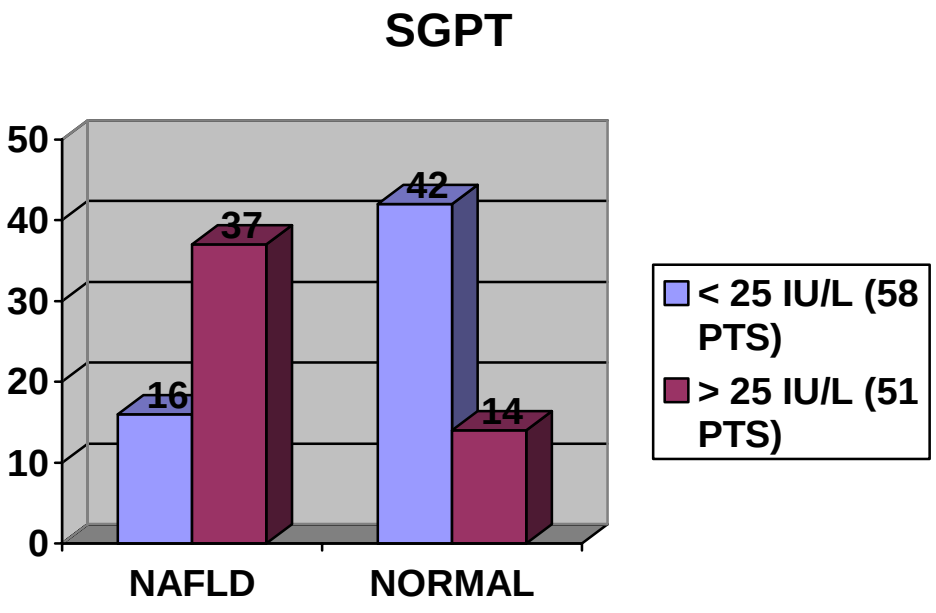
SGPT

Out of the total of 53 persons who had Ultrasongraphically proven fatty liver 26 persons had an SGPT value of more than 25 IU/L and 11 had an SGPT value of more than 35 IU/L.

Out of 56 persons who had normal liver in Ultrasonography 11 persons had an SGPT value of more than 25 IU/L and 3 persons had an SGPT value of more than 35 IU/L.

Mean SGPT Values:

NAFLD group	Normal group	P value
29 ± 28.35 IU/L	17.47 ± 10.02 IU/L	< 0.05



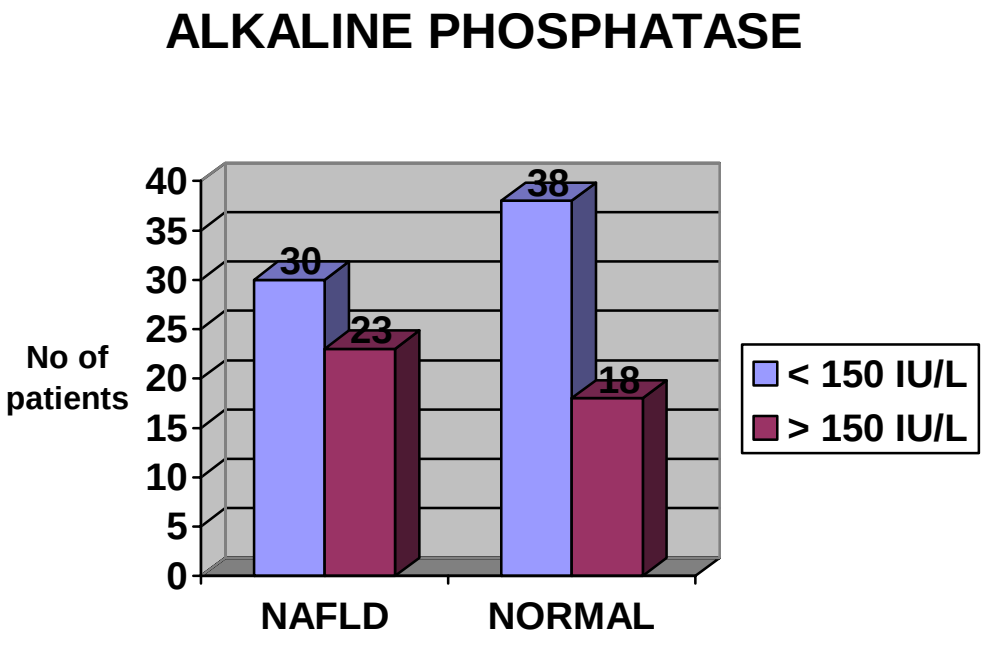
ALKALINE PHOSPHATASE

Out of the total of 53 persons who had Ultrasonographically proven fatty liver 19 (35.84%) persons had a Serum Alkaline Phosphatase value of more than 150 IU/L and 4 individuals had a value of more than 250 IU/l.

Out of a total of 56 persons who had normal liver in Ultrasonography 17 (30.35%) persons had a Serum Alkaline Phosphatase value of more than 150 and 1 person had a Serum Alkaline Phosphatase value of more than 250 IU/l.

Mean Alkaline Phosphatase Values:

NAFLD group	Normal group	P value
123.97 ± 66.13 IU/L	106.52 ± 68.75 IU/L	> 0.05



SERUM BILIRUBIN:

The mean Serum Total Bilirubin in the NAFLD group was 1.10 mg/dl and the Serum Total Bilirubin in the normal group was 0.84 mg/dl. There was no statistically difference in levels of Serum Bilirubin between the two groups.

TOTAL PROTEINS:

The mean value of Total Protein in the NAFLD group was 6.43 gm and in the normal group it was 6.48 gm. There was no statistically difference in levels of Total Protein between the NAFLD and normal liver groups.

FASTING LIPID PROFILE

The lipid done after an overnight fasting of 12 hours included Total Cholesterol, Serum Triglycerides (TGL), Serum High Density Lipoprotein (HDL) and the Low Density Lipoprotein (LDL) value was calculated using the Friedwald formula.

According to the ATP III guidelines for the treatment of lipid disorders, the levels of lipoproteins were considered abnormal if total cholesterol was above 200, if serum triglyceride level was above 150 mg/dl, serum HDL level was below 50 and LDL levels were above 100.

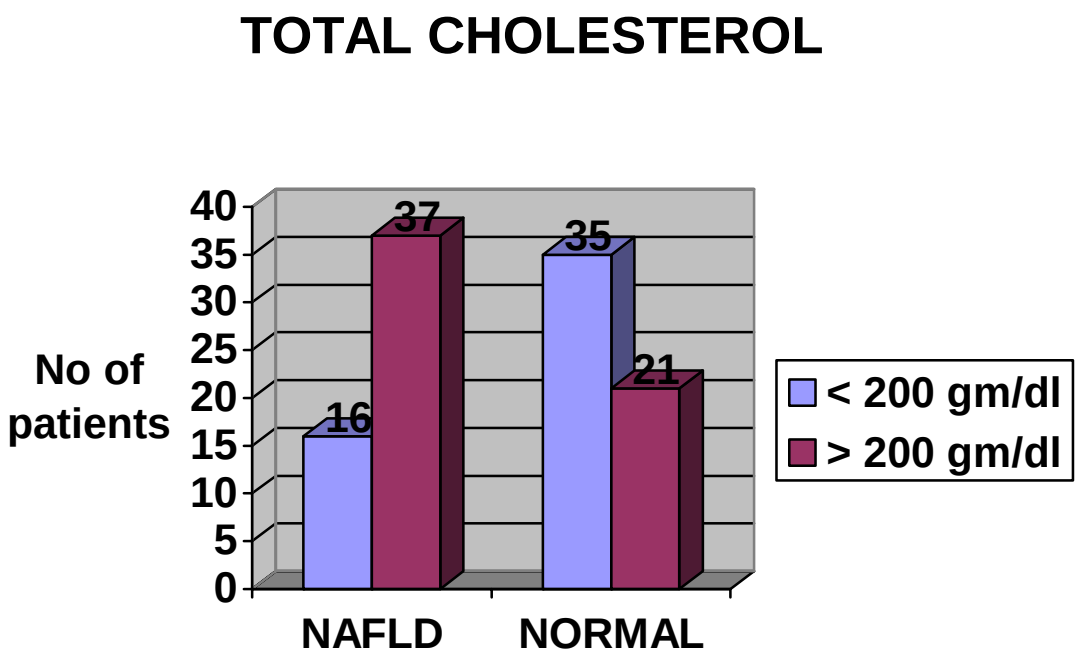
TOTAL CHOLESTEROL:

A total of 58 out of 109 had a high Total Cholesterol value. Among the NAFLD group out of the total 53 patients 37 (69.81 %) had a Total Cholesterol value of more than 200 and among the normal liver group 21 (37.5 %) out of the 56 had a Total Cholesterol value of more than 200.

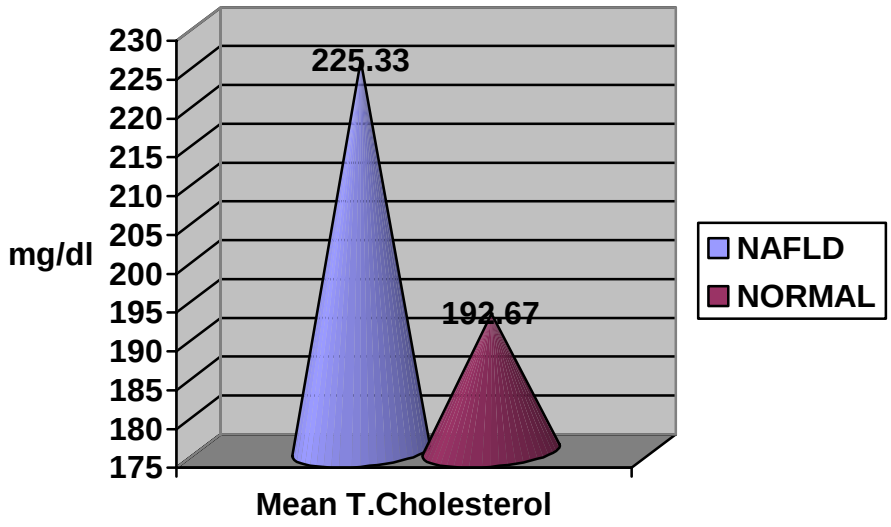
The mean total cholesterol values are as follows:

NAFLD group	Normal group	P value
225.33 ± 43.95 mg/dl	192.67 ± 35.58 mg/dl	< 0.05

TOTAL CHOLESTEROL VALUES



MEAN VALUES:



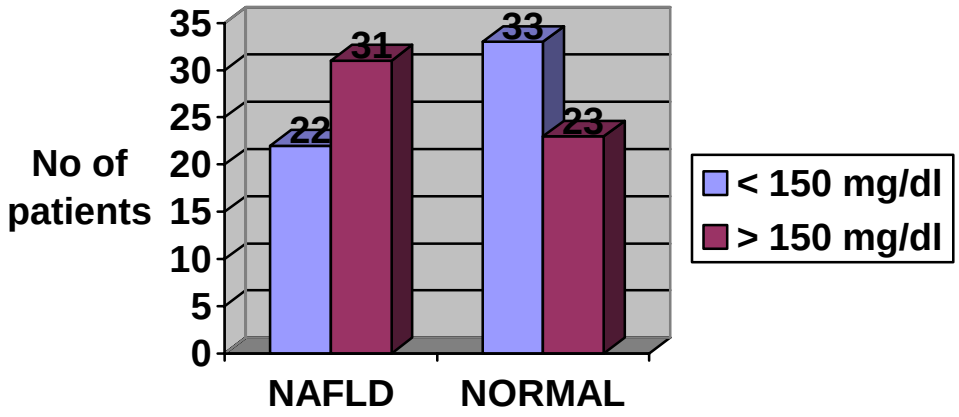
TRIGLYCERIDES:

Out of the 53 patients in the NAFLD group 31 (58.49 %) patients had a triglyceride level of more than 150 mg/dl . Of the 56 patients in the normal liver group 23 (41.07 %) persons had a triglyceride level of more than 150 mg/dl.

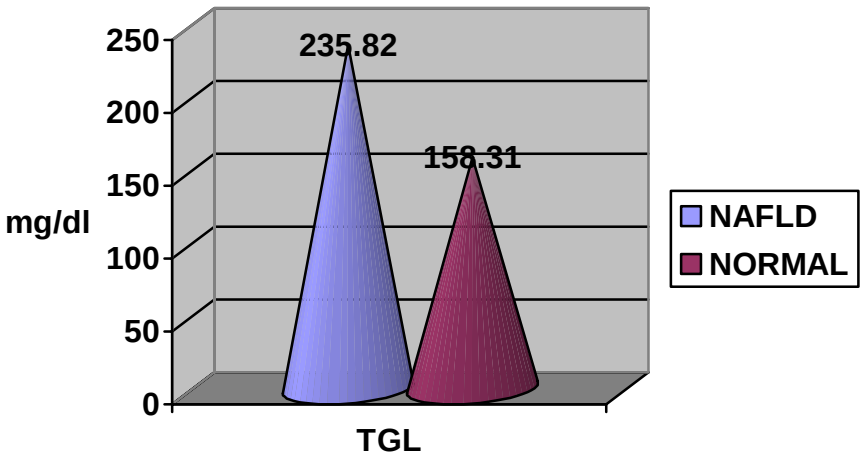
The mean triglyceride levels:

NAFLD group	Normal group	P value
235.82 ± 105.18 mg/dl	155.81 ± 61.08 mg/dl	< 0.05

TGL VALUES



MEAN TGL VALUES:

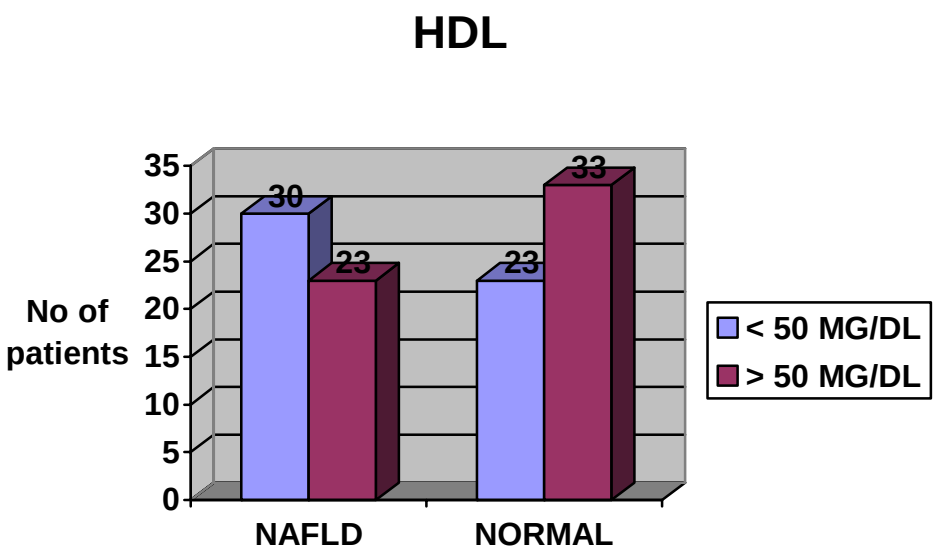


HDL

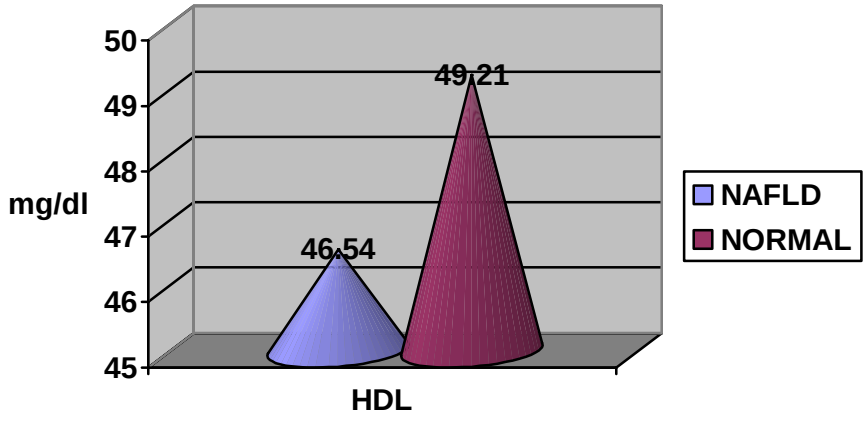
Out of the 53 patients in the NAFLD group 30 (56.6 %) patients had a HDL level of less than 50 mg/dl. Of the 56 patients in the normal liver group 23 persons had a HDL level of less than 50 mg/dl.

The mean HDL levels:

NAFLE group	Normal group	P value
46.24 ± 8.03 mg/dl	49.21 ± 9.93 mg/dl	> 0.05



MEAN HDL VALUES:



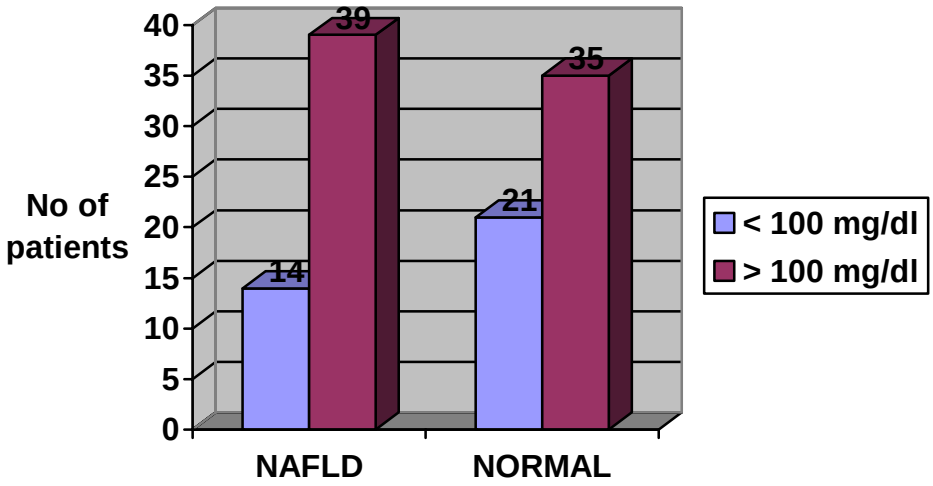
LDL

The LDL levels varied from 52 to 273 mg/dl and a total of 74 patients had LDL levels above 100 mg/dl. In the fatty liver group 39 out of the 53 persons had elevated LDL values above 100 mg/dl. In the normal liver group 35 out of the 66 persons had an elevated LDL level of more than 100 mg/dl.

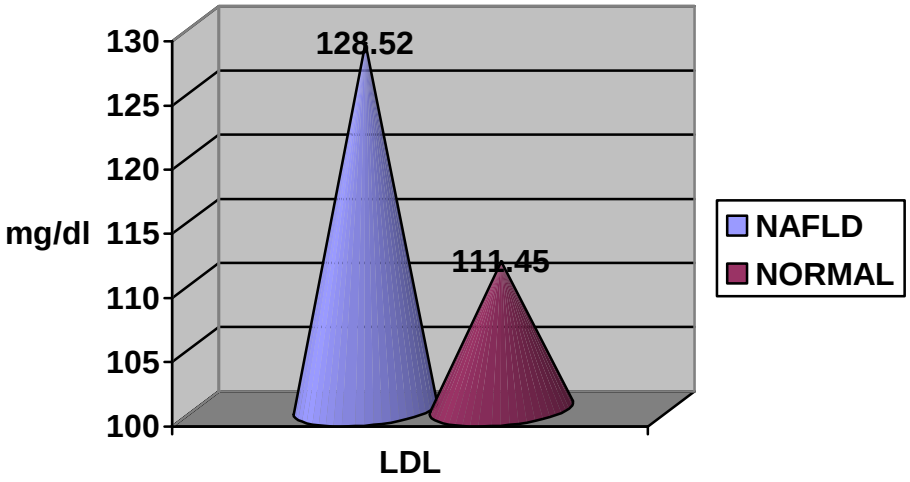
MEAN LDL VALUES:

NAFLD group	Normal group	P value
128.52 ± 41.66 mg/dl	111.45 ± 27.80 mg/dl	< 0.05

LDL



MEAN LDL VALUES



ABSTRACT OF STATISTICAL ANALYSIS

MEAN VALUES:

PARAMETER	**NAFLD GROUP (n=63)**	**NORMAL USG GROUP (n=66)**	**STATISTICAL SIGNIFICANCE AT 5% LEVEL**
DURATION OF DIABETES (yrs)	5.57 ± 3.19	5.48 ± 3.94	No significant difference (P value=0.05)
BMI (kg/m^2)	24.97 ± 3.54	2.29 ± 2.05	**Significant difference present (P value<0.05)**
SGOT (IU/L)	25.02 ± 20.64	18.41 ± 11.97	**Significant difference present (P value<0.05)**
SGPT (IU/L)	29.00 ± 28.35	17.47 ± 10.02	**Significant difference present (P value<0.05)**
ALP (IU/L)	123.97 ± 66.13	106.52 ± 68.75	No significant difference (P value=0.05)
BILIRUBIN (mg/dl)	1.10 ± 1.12	0.84 ± 0.36	No significant difference (P value=0.05)
TOTAL CHOLESTEROL (mg/dl)	225.33 ± 43.95	192.67 ± 35.38	**Significant difference present (P value<0.05**
TGL (mg/dl)	235.82 ± 105.18	155.81 ± 61.08	**Significant difference present (P value<0.05**
HDL (mg/dl)	46.24 ± 8.03	49.21 ± 9.93	No significant difference (P value=0.05)
LDL (mg/dl)	125.82 ± 11.66	111.45 ± 27.80	**Significant difference present (P value<0.05**

ABSTRACT OF DATA: **LIVER ENZYMES :**

ENZYME LEVEL(IU/L)	NAFLD GROUP (53)	NORMAL USG (56)	P VALUE
SGOT < 25 (83)	38	45	> 0.05
>25 (26)	15	11	
SGPT < 25 (58)	16	42	**< 0.05**
> 25 (51)	37	14	
ALP < 150 (68)	30	38	> 0.05
> 150 (41)	23	18	

LIPID PROFILE:

PARAMETER (mg/dl)	NAFLD GROUP (53)	NORMAL USG (56)	P VALUE
TC < 200 (51)	16	35	**<0.05**
> 200 (58)	37	21	
TGL < 150 (55)	22	33	**<0.05**
> 150 (54)	31	23	
HDL < 50 (53)	30	23	>0.05
> 50 (56)	23	33	
LDL < 100 (35)	14	21	> 0.05
> 100 (14)	39	35	

Statistically significant difference at 5 % level (P=0.05) between NAFLD and NORMAL USG groups was present for SGPT, Total Cholesterol and Triglyceride levels. Other parameters did not show any significant difference by comparing the two groups using ‘chi square test’. But mean LDL levels in the NAFLD group were much higher than in that of the normal group.

DISCUSSION

DISCUSSION

A total of 109 patients were included in this study after applying the selection criteria.

Out of the 109 type 2 diabetics included in this study 60 were females and 49 were males, the number of males was lesser than females because alcohol intake was taken as exclusion criteria and so many males got excluded.

Of the 109 diabetics included in this study 53 (48.6 %) of them had ultrasonographically detectable fatty liver, according to several reports the prevalence of fatty liver in Diabetes Mellitus is more than that of the general population, many studies have shown that the prevalence of NAFLD in type 2 Diabetes Mellitus was upto 70 %.

The study of fatty liver in type 2 Diabetes Mellitus

Series	Prevalence of NAFLD by USG (%)
Present (n=109)	48.6 %
Daad H Akbar (n-119)[55]	55%
Gupte P et al (n=100)[56]	49%

The prevalence of fatty liver in this study group is similar to the prevalence observed in other studies.

Out of the 60 female type 2 diabetics 35 (58.33 %) had fatty liver detected by ultrasonography and out of the 49 male type 2 diabetics 17 (34.69 %) had fatty liver. In this study female sex had a higher prevalence of fatty liver (M : F ratio is 1:1.57).

Many studies have shown that female sex has a higher predisposition to the development of fatty liver in the general population. In other studies conducted among type 2 diabetics the prevalence was found to be more among females.

There was no significant variation in the mean age between the NAFLD group and the normal liver group. The mean age of the study population was higher because only persons above the age of 40 years were recruited into the study.

DURATION OF DIABETES:

The mean duration of Diabetes in persons with NAFLD was 5.47 ± 3.19 years and the mean duration of Diabetes in persons with Normal liver in USG was 5.47 ± 3.94 years.

No statistically significant relationship was found between the presence of NAFLD and the duration od Diabetes. The result were similar to the study conducted in Saudi Arabia (Daad H Akbar et al).[55]

BODY MASS INDEX : The mean Body Mass Index in the NAFLD group was significantly higher than that of the normal group. 38 persons had a BMI of more than 25 kg/m^2 and out of them 30 (78.94 %) had NAFLD.

In the study done by Daad H Akbar et al in Saudi Arabia, Obesity was identified as an independent factor for the development of NAFLD.[55]

The number of persons with a BMI of more than 30 kg/m^2 was less compared to studies done in other countries. This is probably due to the low and middle socioeconomic status of the study group. In our study group too the persons with high BMI had prevalence of fatty liver equal to that observed elsewhere.

CLINICAL SYMPTOMS:

Following are the clinical symptoms observed in the group with NAFLD compared to other studies:

Symptoms and signs	Present series (n=53)	Saudi series[55] (n=64)	Virginia series[56] (n=75)
Asymptomatic	52.8 %	80 %	60%
Fatigue	30.1 %	NA	30 %
Right upper quadrant discomfort	20.75 %	17 %	30 %
Jaundice	0 %	NA	NA

Our study had a slight but statistically no significant variation in the incidence of symptoms.

The natural history of NASH in Australia was followed in 42 patients for upto 21 years.[5] Upper abdominal pain was a common reason for presentation. Many studies have shown a high proportion of patients (48 % to 100 %) have no symptoms of liver disease, and a small percentage (especially children[56]) have vague abdominal discomfort or pain in the right upper quadrant[56] or fatigue and malaise.

HEPATOMEGALY:

On clinical examination and Ultrasonogram wise 9 patients (16.18 %) out of 53 in NAFLD group had hepatomegaly. The incidence of hepatomegaly in different studies in diabetes is as follows.

Series	No of patients (%)
Saudi series (2003)[55]	56 out of 64 (88%)
Virginia series (1996)[24]	16 out of 76 (22%)
Vaishnave series (1970)	28 out of 113 (24.7%)
Lal et al (1971)	10 out of 25 (40%)
Present series	9 out of 53 (19%)

There were gross variations in the incidence of hepatomegaly between many groups. None of the patients in the NAFLD group had splenomegaly of ascites. Many studies have shown that the most common finding at initial presentation is asymptomatic hepatomegaly.[4, 5]

LABORATORY INVESTIGATIONS:

The prevalence of NAFLD was not significantly different among different levels of fasting sugar levels in our study.

Many studies have shown that the levels of blood sugar did not have any correlation with development of NAFLD. Moreover HbA1c estimation was done in the Saudi study, [55] and there was no significant relationship between glycemic control and NAFLD.

TRANSAMINASES AND ALKALINE PHOSPHATASE:

There was no statistical difference between the two groups in terms of SGOT and Alkaline Phosphatase elevation in terms of number of persons showing enzyme elevation. But when the mean enzyme values were compared the NAFLD group had a statistically significant higher value than the normal group.

Asymptomatic elevation of transaminases is one of the commonest reported and studied abnormality in NAFLD. The most frequently noted abnormality is two to threefold elevation of levels of ALT and AST in

plasma.[4, 5] V Ness and Diehl[49] found that 19 % of patients (17 of 90) who had liver biopsy for evaluation of chronically elevated plasma levels of ALT and AST in contrast to 7% to 9% of all patients who had liver biopsies for other reasons, nonalcoholic steatosis or steatonecrosis.

Alkaline phosphatase levels are abnormal in fewer than half of patients.[4] Another article has stated that Liver transaminases may be normal, or only marginally elevated (Mofrad et al, 2003).[57] There is poor correlation between biochemistry, ultrasonography and histology, and the entire histological spectrum of NAFLD can be seen in individuals with normal transaminase values (Mofrad et al, 2003).[57]

Some studies have mentioned that Liver enzyme levels in NAFLD patients fluctuate, normal values being present in up to 78% of patients at any one time. When levels are elevated, the increase is mild and often restricted to one or both of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The AST:ALT ratio is usually less than 1, although it may reverse in the presence of cirrhosis.

The SGOT:SGPT ratio in the NAFLD group in this study was 0.8.

In cases with NAFLD the SGOT : SGPT ratio is less than 1 according to literature. In two major studies, [5] levels of ALT were noted to be higher than levels of AST, a pattern that contrasts with that seen in alcoholic hepatitis.

Although values < 1 suggest NAFLD, a ratio of ≥2 is strongly suggestive of alcoholic liver disease.

There was no statistically significant difference in the levels of bilirubin and total proteins between the two groups which were similar to the observations done elsewhere.

FASTING LIPID PROFILE:

Traditionally Total Cholesterol and Triglyceride values were found to be elevated in persons with NAFLD. Our study population consisted of type 2 diabetics and atherogenic dyslipidemias are common among diabetics.

The total Cholesterol, TGL values were significantly higher in terms of number of persons showing elevation and also in terms of the mean values in the NAFLD group. The number of persons showing elevated LDL was similar in both groups but the mean LDL was much higher in the NAFLD group.

Type of Lipid	% of persons	Mean value (mg/dl)
Increased TC	69.8 %	225.3 ± 43.95
Increased TGL	49.5 %	235.82 ± 105.18
Increased LDL	60.6 %	128.52 ± 8.03
Decreased HDL	71.4 %	46.54 ± 41.66

The HDL values were similar in both groups with mean values being marginally lower in the NAFLD group.

The values observed in other studies were as follows. Hyperlipidemis (hypertriglyceridemia, hypercholesterolemia, or both) is another common abnormality and has been reported in 20% to 81% of patients with NAFLD.[2,4,5] Dyslipidemia was present in 65% of cases of NAFLD at the Virginia NAFLD clinic.[24] In another study, Hypertriglyceridemia and fatty liver: clinical diagnosis of fatty liver and lipoprotein profiles in hypertriglyceridemic patients with fatty liver. Most of these patients with fatty liver had hypertriglyceridemia.

Ongoing research has shown that Non alcoholic fatty liver disease has a broad clinical spectrum, different presentations, most of the research has shown that NAFLD has a stable course, some subsets of the NAFLD population might have a progression to severe forms of disease with inflammation termed steatohepatitis and a minority may end up in having cirrhosis. **A significant proportion of patients previously thought to have cryptogenic cirrhosis share many of the clinical and demographic features of NAFLD, suggesting that the etiology of their cirrhosis may be unrecognized NAFLD.** (Powell et al.1990 et al, 1990[5]; Cadwell[23] et al, 1999, Poonawala et al, 2000). Outcomes of NAFLD are different among

different groups and other studies that looked at the outcome of people with NAFLD and Diabetes also report a more aggressive form of disease and higher overall mortality and mortality related to liver disease (Sargin et al, 2003). Older age, increasing obesity, type 2 Diabetes and hypertriglyceridemia appear to be the strongest independent predictors of more advanced disease (Angulo et al, 1999, Dixon et al, 2001).

Follow up of patients with NAFLD has been discussed and monitoring patients with NAFLD is difficult because liver enzyme level tend to improve regardless of whether liver fibrosis worsens or improves.[60] In addition, it may take several decades of monitoring before the development of complications is observed. Therefore, follow-up should be focused on patients who have risk factors for advanced disease.

LIMITATIONS OF THIS STUDY

Although Ultrasound was sensitive for the detection of steatosis its accuracy was greater if more than 30 % of the liver was affected by steatosis. This might lead to an under-estimation of prevalence. But several studies have been conducted with Sonography alone and our study was based on those lines.

Since our study population was derived from the patients attending outpatient clinic, liver biopsy was not feasible and most of the patients were not willing for invasive procedures or inpatient stay. Hence liver biopsy was not carried out. As in all imaging procedures observer error is expected and we tried to minimize this error by review of images by another radiologist.

Moreover certain investigations like insulin levels, C-peptide levels, HbA1c, Transferrin saturation and ferritin levels could not be done in our setup. So we were unable to document hyperinsulinemia etc.

CONCLUSIONS

1. Non alcoholic fatty liver disease is common among the type 2 diabetic population of this region. (Prevalence 48.6% of type 2 diabetics).
2. Female sex has a significantly higher prevalence of non alcoholic fatty liver disease as observed in other geographical regions. (M: F ratio is 1: 1.57).
3. The persons with a higher body mass index are at a greater risk of developing non alcoholic fatty liver disease (78.94 % diabetics with a BMI > 25 kg/m2 had ultrasonographically proven fatty liver)
4. Most of the patients of non alcoholic fatty liver disease are asymptomatic (52.8%). Right Upper Quadrant discomfort and malaise are other symptoms.
5. Hepatomegaly was the commonest physical finding in Non alcoholic fatty liver disease (16.98%). It was found to be present in varying incidences in other studies.
6. No significant relationship was observed between the age of patient, duration of diabetes, fasting blood sugar levels and the presence of Non alcoholic fatty liver disease by ultrasound.
7. There was a significant difference in mean serum transaminase (SGOT, SGPT) levels between the normal and fatty liver groups with

the fatty liver group having higher values. But absolute elevation of transaminases above normal was not seen in many cases.

8. There was no significant relationship observed between Serum Alkalin Phosphatase, Total Bilirubin and Total Proteins and the prevalence of fatty liver by ultrasound.
9. Significantly high Serum Total Cholesterol, Triglycerides and Low Density Lipoproteins were present in persons with fatty liver.
10. No significant correlation was observed between Low Density Lipoprotein levels and the presence of fatty liver in Ultrasound but marginally low mean HDL values were present in the fatty liver group.

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PROFORMA

NAME:			AGE/SEX:	
OCCUPATION:			I.P/O.P NO:	
COMPLAINTS				
RT. UPPER QUADRANT PAIN		ABDOMINAL MASS	JAUNDICE	
ABDOMINAL DISTENSION		OTHERS:		
CLINICAL FINDINGS				
HEPATOMEGALY		SPLENOMEGALY		
OTHERS:				
ALCOHOL INTAKE		YES	NO	
H/O DRUG INTAKE				
AMIODARONE	VALPROATE		CA CHANNEL BLOCKERS	
ANTICANCER DRUS		OTHERS:		
DURATION OF DIABETES:				
HEIGHT(cm):	WEIGHT (kg):		BMI:	
LIVER FUNCTION TESTS				
SGOT	SGPT	SAP	SR.BILIRUBIN	SR.TOTAL PROTEINS
BLOOD SUGAR	RANDOM	FASTING	POSTPRANDIAL	
LIPID PROFILE : TOTAL CHOLESTEROL	VLDL	HDL	LDL	TGL
ULTRASONOGRAM				